Mode of Carcinogenic Action of Pesticides Inducing Thyroid Follicular Cell Tumors in Rodents

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Of 240 pesticides screened for carcinogenicity by the U.S. Environmental Protection Agency Office of Pesticide Programs, at least 24 (10%) produce thyroid follicular cell tumors in rodents. Thirteen of the thyroid carcinogens also induce liver tumors, mainly in mice, and 9 chemicals produce tumors at other sites. Some mutagenic data are available on all 24 pesticides producing thyroid tumors. Mutagenicity does not seem to be a major determinant in thyroid carcinogenicity, except for possibly acetochlor; evidence is less convincing for ethylene thiourea and etridiazole. Studies on thyroid–pituitary functioning, including indications of thyroid cell growth and/or changes in thyroxine, triiodothyronine, or thyroid-stimulating hormone levels, are available on 19 pesticides. No such antithyroid information is available for etridiazole, *N*-octyl bicycloheptene dicarboximide, terbutryn, triadimefon, and trifluralin. Of the studied chemicals, only bromacil lacks antithyroid activity under study conditions. Intrathyroidal and extrathyroidal sites of action are found: amitrole, ethylene thiourea, and mancozeb are thyroid peroxidase inhibitors; and acetochlor, clofentezine, fenbuconazole, fipronil, pendimethalin, pentachloronitrobenzene, prodiamine, pyrimethanil, and thiazopyr seem to enhance the hepatic metabolism and excretion of thyroid hormone. Thus, with 12 pesticides that mode of action judgments can be made, 11 disrupt thyroid–pituitary homeostasis only; no chemical is mutagenic only; and acetochlor may have both antithyroid and some mutagenic activity. More information is needed to identify other potential antithyroid modes of thyroid carcinogenic action. *Key words:* induction of hepatic microsomal enzymes, iodide pump, mode of carcinogenic action, 5′-monodeiodinase activity, pesticides, thyroid follicular cell tumors, thyroid hormone, thyroid peroxidase activity, thyroid-stimulating hormone. *Environ Health Perspect* 106:437–445 (1998). [Online 26 June 1998]
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The modes of action or crucial steps in thyroid follicular cell carcinogenesis include mutagenicity, perturbations in thyroid and pituitary hormones, or a combination of the two (*1*). The role of mutations in thyroid carcinogenesis is demonstrated by the increase in thyroid cancers in rodents treated with internal or external ionizing radiation sources and among people exposed to therapeutic x irradiation, atomic bomb emissions, or the Chernobyl reactor meltdown in the Ukraine (*2,3*). A number of mutagenic chemicals, such as nitrosamines, are also carcinogens in the rodent thyroid (*4*); no chemical is known to be carcinogenic to the human thyroid.

The control of the concentration of thyroid hormone in the blood is regulated by a negative feedback mechanism involving the hypothalamus, the pituitary, and the thyroid (*1*). The hypothalamus releases thyrotropin-releasing hormone (TRH), which stimulates the pituitary to produce thyroid-stimulating hormone (TSH). TSH prompts the thyroid to produce thyroid hormone. Cells in both the hypothalamus and pituitary respond to levels of circulating thyroid hormone. When levels of thyroid hormone are high, the output of both TRH and TSH are low. When levels of thyroid hormone are low, the outputs of TRH and TSH are raised, prompting the thyroid to increase the output of thyroxin (T_4) and triiodothyronine (T_3). The negative feedback loop helps the body to respond to varying demands for thyroid hormone and to maintain hormone homeostasis.

The thyroid gland is capable of meeting physiologic demands for T_4 and T_3 up to a point. However, beyond that point, continuous stimulation of the thyroid may result in changes that could eventually lead to disease, including neoplasia. Persistent elevation of TSH levels stimulates the thyroid gland to deplete its existing stores of thyroid hormone. When the thyroid is not able to keep up with the demand, the follicular cells hypertrophy and cells divide, leading to hyperplasia and nodular hyperplasia. Generally, effects are reversible upon removal of the stimulus, at least early in the process. However, if the stimulus continues, benign and then malignant neoplasms can result. In some cases, chronic stimulation also results in pituitary hyperplasia or tumors involving the cells that produce TSH.

There are many ways chemicals produce antithyroid effects (i.e., perturb thyroid–pituitary homeostasis) that reduce circulating thyroid hormone, increase TSH, and increase thyroid cancer potential in rodents (*5–10*). In the thyroid, these include 1) inhibition of the active transport of inorganic iodide into the follicular cell (iodide pump); 2) inhibition of thyroid peroxidase that converts inorganic iodide into organic iodide and couples iodinated tyrosyl moieties into thyroid hormone; 3) damage to follicular cells; and 4) inhibition of thyroid hormone release into the blood. Outside the thyroid, chemicals can cause 5) inhibition of the conversion of T_4 to T_3 by 5′-monodeiodinase at various sites in the body and 6) enhancement of the metabolism and excretion of thyroid hormone by the liver, largely through the action of uridine diphosphate (UDP) glucuronosyltransferase.

Recently, the U.S. Environmental Protection Agency (EPA) developed a science policy for the assessment of thyroid follicular cell tumors and concluded that rodent thyroid tumors were relevant to the assessment of carcinogenicity in humans (*11,12*). This paper summarizes data within the EPA Office of Pesticide Programs (OPP) files on selected pesticides that have been observed to induce thyroid follicular cell tumors in rodents, in accordance with the guidance in the EPA science policy on thyroid tumors and generic procedures for the assessment of chemicals for carcinogenicity (*13,14*). Rodent thyroid C cell tumors are excluded from the review. Included are determinations of potential mutagenic and antithyroid modes of action. Comparisons are made between the pesticide responses and those of pharmaceuticals from the files of the Food and Drug Administration (FDA) and chemicals tested by the National Toxicology Program/National Cancer Institute (NTP/NCI).

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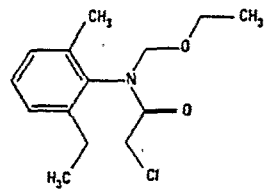
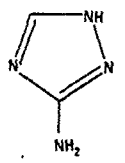


Acetochlor



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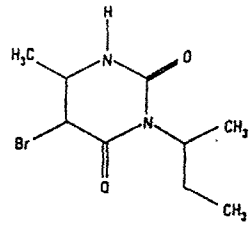
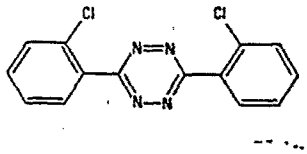
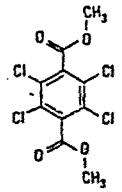


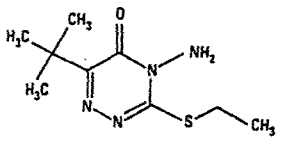
Bromacil



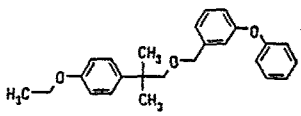
Clofentezine



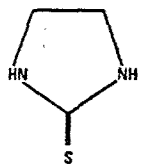
2,3,5,6-Tetrachloro-1,4-benzenedicarboxylic acid dimethyl ester



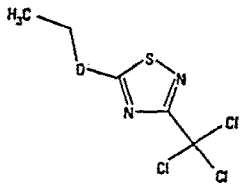
Ethiozin



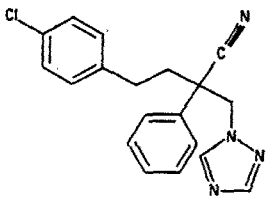
Ethofenprox



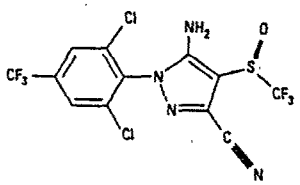
Ethylene thiourea



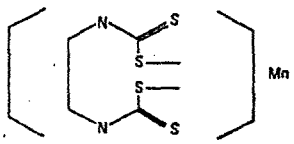
Etridiazole



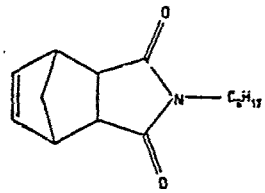
Fenbuconazole



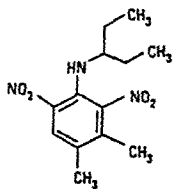
Fipronil



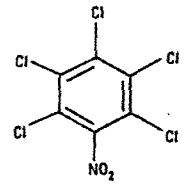
Mancozeb:
complex of Zn and Maneb (shown)



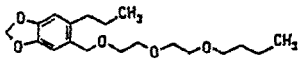
N-Octyl bicycloheptene dicarboximide



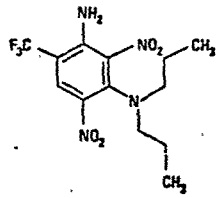
Pendimethalin



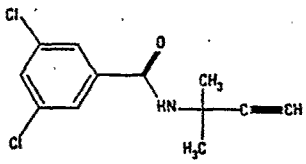
Pentachloronitrobenzene



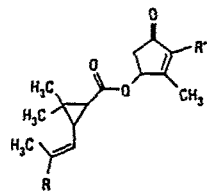
Piperonyl butoxide



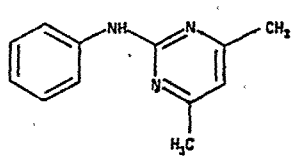
Prodiamine



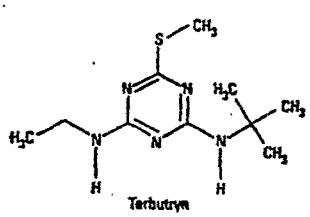
Pronamide



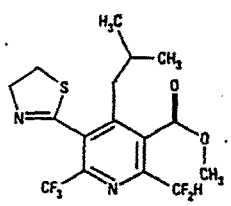
Pyrethrins:
Pyrethrin I: R = CH_3; R' = $CH_2CH=CHCH_2$
Pyrethrin II: R = $COOCH_3$; R' = $CH_2CH=CHCH_2$
Cinerin I: R = CH_3; R' = $CH_2CH=CHCH_3$
Cinerin II: R = $COOCH_3$; R' = $CH_2CH=CHCH_3$
Jasomolin I: R = CH_3; R' = $CH_2CH=CHCH_2CH_3$
Jasomolin II: R = $COOCH_3$; R' = $CH_2CH=CHCH_2CH_3$



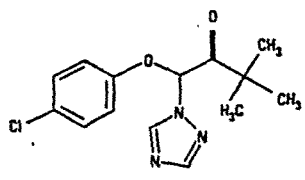
Pyrimethanil



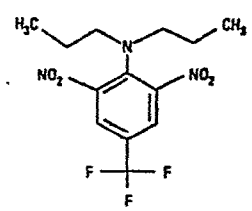
Terbutryn



Thiazopyr



Triadimefon



Trifluralin

Figure 1. Structures of 24 pesticides that produced thyroid tumors.

Methods

The OPP data files at the EPA were systematically searched for pesticides that have been observed to induce effects on the thyroid (i.e., biologically relevant changes in thyroid and pituitary hormones, indications of thyroid growth, and various other effects on thyroid follicular cells). The review was primarily focused on pesticides that induce thyroid follicular cell tumors in at least one sex of one species. The search also yielded identification of pesticides that exhibited some antithyroid activity but were not observed to induce thyroid follicular cell tumors. For the newer pesticides in the files, specific thyroid function studies were available; the database for these chemicals was robust and easily summarized. For the older pesticides, relevant mechanistic data were scattered throughout the file, and pertinent information needed to be pieced together.

Carcinogenicity data were extracted from 2-year bioassays, mostly conducted according to EPA testing guidelines. The carcinogenicity data for amitrole is an exception in that none of the studies in themselves were conducted in accordance with EPA guidelines. These studies were thus assessed as a group (i.e., weight of the evidence). Mutagenicity data were mostly taken from unpublished studies submitted to OPP that were considered acceptable. Occasionally, studies published in the open literature were also available in the OPP files. However, an extensive search of the published literature was not conducted. Published papers were used where data were absent, but except for a few pesticides such as amitrole and ethylene thiourea, little was found (*15–35*). Unpublished studies from OPP files are not cited in the bibliography.

Results and Discussion

Thyroid Tumors

Number of chemicals positive. Approximately 240 pesticides have been screened for potential carcinogenicity in rodents by the OPP. The OPP Cancer Peer Review Committee (CPRC) has completed an in-depth review on 167 of these. Thirty-seven of the 240 pesticides induce effects on thyroid follicular cells. Twenty-seven of these have been determined by the CPRC to induce thyroid follicular cell tumors in acceptable studies in rodents: 24 produce tumors in appropriate chronic tests in rodents; 3 induce these tumors only at excessively toxic dose levels (alachlor, carbaryl, and oryzalin) and are not considered in this review. Four others were determined by the CPRC not to induce thyroid follicular cell tumors in submitted studies, although they exhibit some antithyroid activity. Of the remaining 6 pesticides that induce effects on thyroid follicular cells but have not yet been through CPRC review, preliminary evaluation indicates that 4 may also induce thyroid follicular cell tumors and 2 demonstrate some antithyroid activity but not tumors in submitted studies. Thus far, the CPRC has determined that 10% of pesticides (24/240) produce thyroid follicular cell tumors in chronic studies at appropriate dose levels. If one combines the 24 thyroid carcinogens identified by the CPRC with the 4 that have yet to be reviewed, then up to 12% (28/240) of the total number of pesticides may produce thyroid tumors in rodents. In comparison to pesticides, the proportion of pharmaceuticals evaluated by the FDA and the proportion of chemicals tested by the NTP/NCI that are positive for thyroid tumors in rodents are 18/282 (6%) and 21/460 (5%), respectively (*36,37*).

Cursory review of the 240 pesticides evaluated by the OPP reveals that thyroid follicular cell tumors are among the most common tumor types induced by pesticides, with the liver being the most common. Thyroid tumors are second, followed closely by lung, mammary, and Leydig cell tumors. Thyroid tumors rank between second and eighth in the FDA database and between fifth and eighth in the NTP/NCI database, depending on sex and species. Only two of the pesticides reviewed in this paper are also included in the NTP/NCI database, ethylene thiourea and pentachloronitrobenzene, although the latter chemical was not positive for thyroid tumors in the study by the NTP/NCI. About 20% of the drugs in the FDA data set are also included in the NTP/NCI database (*36*). Some of the listed thyroid carcinogens among the pharmaceuticals may have induced thyroid C cell and not follicular cell tumors.

For the purpose of this review, only the 24 chemicals that have been through the CPRC process and have been determined to induce thyroid follicular cell tumors at appropriate dose levels are discussed. Figure 1 shows the chemical structures of these chemicals.

Grouping of pesticide responses. An examination of the tumor data for the 24 pesticides provides some interesting differences in thyroid tumor incidence. By looking at the combined benign and malignant thyroid tumor responses at the highest dose administered to male rats, two groups are apparent (Table 1). Group 1 includes three pesticides that induce a high incidence of thyroid tumors (≥0.48) at relatively low daily doses: amitrole, ethylene thiourea, and mancozeb, which is metabolized to ethylene thiourea. The 21 pesticides in Group 2 induce lower thyroid tumor incidences in male rats (≤0.39) at higher dose levels than in Group 1 except for two members of the group. For Group 1, significantly increasing dose trends are observed for the incidence of adenomas, carcinomas, and combined adenomas/carcinomas for all three chemicals, and most pairwise comparisons between treatment groups and controls also demonstrate significant differences. Group 2 commonly induces statistically significant increases in dose-related trends in tumor incidence, but pairwise comparison increases are less common. Adenomas are more abundant than carcinomas and contribute the most towards the significance of the combined adenoma and carcinomas.

Species and sex differences. Among the pesticides, 22 of the 24 induce thyroid tumors only in rats; none induces tumors only in mice. Increased thyroid tumor incidence is observed in both mice and rats for only two pesticides, amitrole and ethylene thiourea, members of the first group of pesticides that are potent inducers of thyroid tumors (Table 2). With the FDA pharmaceuticals, 15 of 18 chemicals are positive for thyroid tumors only in the rat, 1 is positive only in the mouse, and 2 are positive in both rats and mice (*36*). From the NTP/NCI database, 9 of 21 induce thyroid tumors only in rats, 5 only in mice, and 7 in both rats and mice (*37*). The species difference in the three databases has not been explained. Adult rats and mice both lack thyroid hormone binding globulin, the specific high-affinity serum carrier protein that exists in humans (*38*). The absence of the carrier protein results in a greater proportion of free thyroid hormone in the serum, more readily available to metabolism and excretion. With a shorter half-life of thyroid hormone, rodents should be more sensitive than humans to chemically induced thyroid–pituitary disruption and should have increased susceptibility to developing thyroid tumors. However, this does not explain the differences in response between rats and mice.

Male rats are frequently more sensitive to thyroid carcinogens than females, both with respect to the proportion of chemicals that induce thyroid tumors and to tumor incidence. In keeping with this, TSH levels are higher in male rats than in female rats (*11*). Eight of the 24 pesticides reviewed in this

Table 1. Pesticide groupings: range of highest dose tested and thyroid tumor incidence in male rats

Pesticide group	Dose (mg/kg/day)	Incidence of benign and malignant tumors
Group 1[a]	3.5–30.9	0.48–0.91
Group 2[b]	13–1000 (median 143)	0.08–0.39

[a]Includes amitrole, ethylene thiourea, and mancozeb.
[b]Includes 21 pesticides.

Table 2. Pesticides producing significant tumor incidences according to sex and species

Pesticide	Thyroid	Liver	Other tumor sites
Acetochlor	Rmf	M	Bone (R), glandular stomach (R), nasal cavity (R), lung (M)
Amitrole	Rmf, Mmf	M	Pituitary (R)
Bromacil	Rm	M	Thyroid C cell (R)
Clofentezine	Rm		
DCPA	Rmf	M, R	
Ethiozin	Rmf		
Ethofenprox	Rmf		
Ethylene thiourea	Rmf, Mmf	M	Pituitary (M)
Etridiazole	Rm	R	Bile duct (R), mammary gland (R), testis, (R)
Fenbuconazole	Rm	M	
Fipronil	Rmf		
Mancozeb	Rmf		
N-OBHD	Rm	M	
Pendimethalin	Rmf		
Pentachloronitrobenzene	Rmf		
Piperonyl butoxide	Rmf	M	
Prodiamine	Rmf		Pancreas islet cell (R), subcutaneous (M)
Pronamide	Rmf	M	Testis (R)
Pyrethrins	Rmf	R	Parathyroid (R)
Pyrimethanil	Rmf		
Terbutryn	Rm	R	Mammary gland (R), testis (R)
Thiazopyr	Rm		Kidney (R)
Triadimefon	Rmf	M	
Trifluralin	Rm		Urinary bladder (R), kidney (R)

Abbreviations: R, rat; M, mouse; f, female; m, male; DCPA, 2,3,5,6-tetrachloro-1,4-benzenedicarboxylic acid dimethyl ester; N-OBHD, *N*-octyl bicycloheptene dicarboximide.

paper induce thyroid tumors in males only, none is positive in females only, and the rest are positive in both sexes (Table 2). In the FDA pharmaceutical database, 8 chemicals out of 18 are positive for thyroid tumors in male rats only, 1 is positive in females only, and 8 are positive in both male and female rats (*36*). Among the NTP/NCI chemicals, 2 out of 21 are positive in male rats only, 1 is positive in females only, and 6 are positive in both male and female rats (*37*). In contrast to rodents, human females more often develop thyroid cancer than do males, but there is no difference in TSH levels (*11,39*).

Other Tumors

Increases in pituitary tumors (that may involve TSH-secreting cells and be mechanistically related to thyroid tumors through an antithyroid chemical action) in rats or mice are observed only with two pesticides, again members of the first group, amitrole and ethylene thiourea (Table 2). Two of the FDA pharmaceuticals show increases in both thyroid and pituitary tumors: the antihypertensive atenolol produces both tumors in male rats, and a cardiotonic produces thyroid tumors in male and female rats and mice and pituitary tumors in female mice (*36*). Among the NTP/NCI chemicals, iodinated glycerol increases thyroid tumors in male rats and pituitary tumors in female mice; ethylene thiourea is a chemical in common with the EPA database (*37*).

Sixteen of the 24 pesticidal thyroid carcinogens induce tumors in at least one other site (not including the pituitary). By far the most common site is the liver, a finding shared with pharmaceuticals and NTP/NCI chemicals. Ten pesticides induce liver tumors in mice; four induce liver tumors in rats; and one induces liver tumors in both mice and rats. An association between the occurrence of thyroid and liver tumors has been observed in rodent cancer studies (*40,41*); however, the basis for this relationship is not fully understood, although induction of liver microsomal enzymes may play a role in some cases. With the 24 pesticides, there is also a preponderance of tumors in endocrine organs, particularly in the rat, including glandular stomach, mammary gland, parathyroid, pancreas islet cell, testis, and thyroid C cell. Pharmaceuticals also frequently induce tumors in endocrine organs in the rat (*36,42*), in contrast to chemicals in the NTP/NCI database, where endocrine tumors are less frequently noted (*37*). Finally, several other tumors were observed with the 24 pesticides reviewed here. These are found mainly in the rat and include bile duct, bone, kidney, lung, nasal cavity, subcutaneous, and urinary bladder. Some of these tumors are rare, and incidences are numerically but not statistically significantly increased in comparison to the control.

Mutagenic Activity

All 24 pesticides have been studied in at least one acceptable *in vitro* or *in vivo* gene mutation test, and all except ethiozin have been tested for chromosome aberrations (Table 3). The results of this testing indicate that mutagenicity does not seem to be a strong mechanistic influence for tumor development. Only acetochlor, ethylene thiourea, and etridiazole produce any positive responses across both the gene mutation and chromosomal aberration end points. Acetochlor is unique in that it shows positive or weakly positive effects for bacterial and mammalian cell gene mutations and for chromosome aberrations, both *in vitro* and *in vivo*. However, the data on acetochlor is mixed with both positives and negatives and the bacterial gene mutation test is positive only in one strain (*43*). Ethylene thiourea produces negative and weakly positive bacterial mutation, positive and negative mammalian cell gene mutation, and negative *in vitro* and positive *in vivo* chromosome aberration tests. Etridiazole induces positive effects in bacterial gene and *in vitro* structural chromosome mutation tests. Fifteen pesticides produce only negative results across both the gene mutation and chromosomal aberration end points, and an additional three produce all negative results except a mixed response in one of the gene mutation tests. These results indicate that for the 24 pesticides, except for possibly acetochlor, ethylene thiourea, and etridiazole, the thyroid tumors are likely to have been induced through a mode of action other than mutation.

Most FDA pharmaceuticals, except antineoplastics and a few others, are like pesticides in that generally they are not mutagenic (*42*). In contrast with these two groups of chemicals, a much higher proportion of chemicals in the NTP/NCI thyroid carcinogen database show mutagenic effects. This is especially true for the aromatic amines, including such compounds as 3-amino-4-ethoxyacetanilide, C.I. basic red 9 monochloride, and 4,4′-thiodianiline (*1*). Not uncommonly, these chemicals produce both gene and chromosomal mutations in cell culture test systems and, in some cases, in chromosome aberration tests *in vivo*.

Perturbations in Thyroid–Pituitary Functioning

Several crucial pieces of information are combined to determine whether a chemical produces thyroid tumors by interfering with thyroid–pituitary homeostasis (*11*). Such substances induce increases in thyroid cell growth and perturb thyroid and pituitary hormones, effects that are reversible upon cessation of dosing. Identification of a specific site of antithyroid action is needed, along with correlations among chemical doses that perturb thyroid–pituitary functioning and thyroid tumors.

Increased thyroid cell growth and hormone changes. Data on 19 of the 24 pesticides indicate whether or not there is induction of

thyroid growth following chemical administration (Table 4). Follicular cell hyperplasia is observed in 15 cases, cellular hypertrophy in 13, and an increase in thyroid gland weight in 14. Ten chemicals affect all three indicators of thyroid cell growth, and another four affect two thyroid cell growth indicators. Measures of increased thyroid growth are usually gathered as part of standard subchronic and chronic toxicity studies. Thus, such findings (both positive and negative) are usually reported for both sexes of several species, usually rats, mice, and dogs; however, when information is only available for one animal group, it is usually for male rats.

Data are available on either thyroid or pituitary hormones for 17 of the 24 pesticides (Table 4). No hormonal data exist for 7: etridiazole, *N*-octyl bicycloheptene dicarboximide (N-OBHD), piperonyl butoxide, pyrethrins, terbutryn, triadimefon, and trifluralin. With the exception of ethylene thiourea and mancozeb, which have data on several species, the majority of the hormonal data are available for both sexes of rats; however, since studies measuring hormonal levels are often separate from the standard subchronic and chronic studies, several of the studies were conducted only with male rats because males tend to be more sensitive than females with regard to effects on thyroid hormones. Eleven pesticides induce decreases in T_4 and/or T_3 and increases in TSH (all three parameters are not necessarily measured in the same study). A reduction in both serum T_4 and T_3 levels is noted in at least one study for 7 pesticides and a reduction in only serum T_4 levels is reported in at least one study for 6 pesticides. Data include an increase in serum TSH levels in at least one study for 12 pesticides and no change in TSH levels for 4 chemicals. Some of the pesticides appear to affect either one or both of the thyroid hormones but not the pituitary hormone, and vice versa.

For evaluation of thyroid growth and especially thyroid–pituitary hormone status, timing of sampling is important because of the compensatory action of homeostatic mechanisms. As a result, it is sometimes difficult to discern changes after compensation ensues. Histological and hormonal data submitted to the OPP for the pesticides that induce thyroid tumors were collected over a wide variety of time periods, doses, species, and methods. In many cases, careful timing of the measurements was either not done or not reported. Therefore, some data may be misleading or limited. All but one of the 19 studied chemicals (bromacil) showed at least one of the five indications of increased thyroid growth or hormone perturbation, and 15 chemicals showed multiple effects indicative of an antithyroid effect.

The pesticide hormonal data were examined for a potential correlation between degree of disruption in hormone measurements and extent of the carcinogenic effect. Only nine pesticides have hormonal data that are amenable for comparison purposes: acetochlor (*43*), amitrole, clofentezine, ethiozin, ethylene thiourea, fipronil, pendimethalin, pronamide, and thiazopyr. Each of these chemicals has hormonal data

Table 3. Mutagenic activity of pesticides that induce thyroid tumors

	Gene mutation test		Chromosome aberration test	
Pesticide	Bacterial	Mammalian	*In vitro*	*In vivo*
Acetochlor	+?/–[a]	+/+/–	+/–	–/–/–/–/+
Amitrole	–			–
Bromacil	–	+/–		–
Clofentezine	–	–		–
DCPA		–	–	
Ethiozin	w/–/–			
Ethofenprox	–/–		–/–	–
Ethylene thiourea	w/–	+/–	–	+
Etridiazole	+	–	+	–
Fenbuconazole		–		–
Fipronil	–/–	–/–	–	
Mancozeb	–			–
N-OBHD	–	w	–	
Pendimethalin	+/–/–/–/–	–	–	–
Pentachloronitrobenzene	–	–	+	
Piperonyl butoxide	–	+/–	–	
Prodiamine	–	–	–	
Pronamide	–	–	–	–
Pyrethrins	–		–	
Pyrimethanil	–/–		–	–
Terbutryn	–		–/–	
Thiazopyr	–			–
Triadimefon	–		–	
Trifluralin	–	–		–/–/–

Abbreviations: +, positive; -, negative; w, weakly positive; ?, positive in only one strain; DCPA, 2,3,5,6-tetrachloro-1,4-benzenedicarboxylic acid dimethyl ester; N-OBHD, *N*-octyl bicycloheptene dicarboximide.
[a]Results are from separate studies.

Table 4. Antithyroid data for pesticides that induce thyroid tumors

	Indication of thyroid cell growth			Hormone changes	
Pesticide	Cellular hypertrophy	Hyperplasia	Increase in thyroid weight	Decrease in thyroid hormone	Increase in TSH
Acetochlor			Yes	Yes[a]	Yes
Amitrole	Yes	Yes	Yes	Yes[b]	Yes
Bromacil	No	No	No	No[c]	No
Clofentezine	Yes	Yes	Yes	Equivocal[d]	Yes
DCPA	Yes	Yes	Yes	Yes[e,f]	No
Ethiozin	No	Yes	Yes	Yes[b]	Yes
Ethofenprox	Yes	No	Yes	Yes[b]	
Ethylene thiourea	Yes	Yes	Yes	Yes[b]	Yes
Fenbuconazole	Yes	Yes	Yes	Yes[e,f]	Yes
Fipronil	Yes	No	Yes	Yes[e,g]	Yes
Mancozeb	Yes	Yes	Yes	Yes[b]	Yes
Pendimethalin	Yes	Yes	Yes	Yes[b]	Yes
Pentachloronitrobenzene	Yes	Yes	Yes	Yes[b]	Yes
Piperonyl butoxide	No	Yes	No		
Prodiamine	No	Yes	No	No[c]	No
Pronamide	Yes	Yes	Yes	Yes[e,f]	No
Pyrethrins	No	Yes	No		
Pyrimethanil	Yes	Yes	No	Yes[e,f]	Yes
Thiazopyr	Yes	Yes	Yes	Yes[e,g]	Yes

Abbreviations: TSH, thyroid-stimulating hormone; DCPA, 2,3,5,6-tetrachloro-1,4-benzenedicarboxylic acid dimethyl ester; T_4, thyroxin; T_3, triiodothyronine.
[a]Reduction in serum T_3 only.
[b]Reduction in both serum T_4 and T_3.
[c]No change in either serum T_4 or T_3.
[d]Equivocal changes in serum T_4 and T_3.
[e]Reduction in serum T_4 only.
[f]No change in serum T_3.
[g]Equivocal changes in serum T_3.

from at least one 28-day study, as well as tumor responses in a chronic study. Although data are difficult to compare due to variability in study protocols, generally, it appears that compounds in the group of pesticides that induce higher tumor incidences also lead to more significant deviations in hormone levels (i.e., amitrole and ethylene thiourea).

Site of action. No pesticide has been investigated for all the potential antithyroid sites of action: inhibition of iodide uptake into the thyroid, thyroid peroxidase inhibition, damage to thyroid follicular cells, inhibition of thyroid hormone release from the thyroid, inhibition of 5'-monodeiodinase activity, and enhancement of thyroid hormone metabolism and excretion by the liver (*1*). Most attention has focused on assessing one or possibly two possible sites of action. No studies on pesticides have reported chemical damage to thyroid cells or inhibition of thyroid hormone release, although only a limited number of chemicals in the literature seem to affect these processes, such as lithium and excess iodide (*44*) and ionizing radiation and polychlorinated biphenyls (*2,45*), respectively.

Ten pesticides have been investigated regarding an intrathyroidal site of action, mainly in male rats (Table 5). Amitrole, ethylene thiourea, and mancozeb inhibit thyroid peroxidase (*46–48*). These three chemicals are members of the group of pesticides that produce high thyroid tumor incidences (Group I, Table 1). They are also the chemicals that seem to lead to greater perturbations in thyroid and pituitary hormone levels, produce significant indications of thyroid cell growth, and induce pituitary tumors that may be related to thyroid–pituitary disruption (*35,49*). The responses to these pesticides in rodents are similar to responses to other thyroid peroxidase inhibitors, such as pharmaceuticals used for treatment of hyperthyroidism (e.g., propylthiouracil, methimazole) and various sulfonamides (*50–53*).

Both mancozeb and its metabolite ethylene thiourea are thionamides, a group of chemicals with antithyroid activity (Fig. 1) (*44*). Other classes of compounds among the thyroid peroxidase inhibitors include certain aromatic amines and polyhydroxyphenols. The NTP/NCI database contains several thionamides and many aromatic amines; chronic rodent testing of two of the polyhydroxyphenols surprisingly failed to yield thyroid tumors or effects (*54,55*).

Five pesticides are reported to inhibit iodide uptake into the thyroid (Table 5); however, for two of these, ethiozin and pentachloronitrobenzene, the data are incomplete and are difficult to interpret. For amitrole, ethylene thiourea, and mancozeb, it is not known whether the reduced iodide uptake is due to a specific block in the active transport of inorganic iodide into the cell (iodide pump) or whether it is simply a manifestation of the inhibition of thyroid peroxidase, due to the fact that the iodide is not trapped within the cell in an organic form (*15,46–48*). Further work is required to differentiate these possibilities. Inhibition of the iodide pump is not a common manifestation of chemical toxicity; it is seen with certain anions like perchlorate and thiocyanate, some of which are used clinically (*44*).

For five pesticides, there is actually an increase in iodide uptake into the thyroid. By itself, an increase in iodide uptake is consistent with an increase in thyroid hormone synthetic activity. In keeping with this, the increase in iodide uptake is accompanied by an increase in thyroid peroxidase activity for fipronil. Increases in iodide uptake are undoubtedly a reflection of enhanced hepatic metabolism and excretion of thyroid hormone, which results in an enhancement of thyroid hormone synthetic activity.

Most studies on pesticides that investigated potential sites of action outside of the thyroid gland were conducted with male rats. Evidence suggests that a potential site of antithyroid action for the bulk of the pesticides may be the liver. With the exception of amitrole and terbutryn, there is histological evidence that all of the pesticides under review induce hepatocellular hypertrophy, increase liver weight, and/or increase smooth endoplasmic reticulum in the liver of at least one species. Other indications of potential hepatic activity are available for 14 pesticides (Table 6). There are increases in mixed-function oxidase activity in at least one species for 9 pesticides and increases in biliary flow and/or biliary excretion for seven chemicals. Specific data bearing on pesticidal influence on thyroid hormone metabolism—increase in T_4 serum clearance and/or increase in T_4 UDP-glucuronosyltransferase activity—are present for nine agents. Terbutryn has no data bearing on a hepatic site of action. Seven agents, namely clofentezine, fenbuconazole, pendimethalin, pentachloronitrobenzene, prodiamine, pronamide, and thiazopyr, show increases in at least two of the parameters, consistent with an enhancement of hepatic metabolism and excretion of thyroid hormone.

Unlike the thyroid peroxidase inhibitors, inducers of liver microsomal enzymes are not necessarily confined to a limited number of chemical classes (Fig. 1). Acetochlor is related to alachlor (*56*), but alachlor was not included in the list because thyroid tumors occurred only at doses producing excessive toxicity. Like pesticides, the liver seems to be a common site of action for a wide array of pharmaceuticals, including compounds such as simvastatin and, most likely, its analog fluvastatin; doxylamine; etretinate; nicardipine; oxazepam; and spironolactone (*36,57–60*).

Table 5. Intrathyroidal site of action for pesticides that induce thyroid tumors

	Biochemical activity	
Pesticide	Iodide uptake	Thyroid peroxidase
Amitrole	⇓R	⇓R
Clofentezine	⇑R; ⇑M	
Ethiozin	w⇓R	
Ethylene thiourea	⇓R	⇓R; ⇓P
Fipronil	⇑R	⇑R
Mancozeb	⇓R	⇓R
Pendimethalin	⇑R	= R
Pentachloronitrobenzene	⇓R	
Pyrimethanil	⇑R	= R
Thiazopyr	= R	

Abbreviations: M, mouse; P, primate; R, rat; w, weak; ⇓, decrease; ⇑, increase; –, no change.

Table 6. Potential hepatic site of action for pesticides that induce thyroid tumors

	Increase in				
Pesticide	Mixed function oxidase activity	Biliary flow	Biliary excretion	T_4 Serum clearance	UDPGT activity
Acetochlor					Yes
Clofentezine	M,R	w, Yes	w, Yes	No change	Yes
Ethylene thiourea	M, not R				
Fenbuconazole	M,R	Yes	Yes	Yes	Yes
Fipronil				Yes	
Pendimethalin		Yes	Yes		w, Yes
Pentachloronitrobenzene		Yes	Yes	Yes[a]	
Piperonyl butoxide	M				
Prodiamine	R		No change	T_3	Yes
Pronamide	R	Yes	Yes		
Pyrimethanil					Yes
Thiazopyr	R		Yes		Yes
Triadimefon	D				
Trifluralin	M				

Abbreviations: D, dog; M, mouse; R, rat; T_4, thyroxin; UDPGT, uridine diphosphate glucuronosyltransferase; w, weak effect; T_3, triiodothyronine.
[a]Biliary T_4 clearance.

Many classes of chemicals induce hepatic microsomal enzymes. UDP-glucuronosyl transferase activity toward various substrates is induced by disparate chemical structures, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, 3-methylcholanthrene, and other polyaromatic hydrocarbons; phenobarbital; polychlorinated biphenyls; pregnenolone-16 α–carbonitrile; and clofibrate. Induction by each of these chemicals results in increased transferase activity toward T_4 (*61–66*).

Little attention has been given to the determination of whether pesticides block the conversion of T_4 to T_3 by inhibiting 5′-monodeiodinase activity in various tissues. Studies have been conducted on only two pesticides, amitrole and thiazopyr. Neither showed a reduction in 5′-monodeiodinase activity. In fact, amitrole enhances T_4 inner ring 5-deiodination, which leads to an increase in reversed T_3 production (*67–69*).

Reversibility of effects. Partial or complete reversibility of effects following cessation of short-term treatment provides evidence of a nonself-perpetuating process, a manifestation consistent with a thyroid–pituitary imbalance mode of action (*1*). Data are available for 10 pesticides, including measurements of reversibility of thyroid growth indicators (cellular hypertrophy, hyperplasia, thyroid gland weight), decreases in T_4 or T_3, or increases in TSH (Table 7). The time periods for the studies range from 2 to 50 weeks of treatment, with 2–16 weeks of recovery. In general, each studied parameter returns toward normal when pesticide dosing stops. Only for ethiozin and thiazopyr is a lack of reversibility of some measured parameters indicated under the conditions of testing.

Dose correlations. Dose correlations refer to associations between doses of a chemical that induce relevant antithyroid effects and those that produce thyroid follicular cell tumors. They are important elements in assessing quantitatively potential risks (*11*). Such data are limited for the 24 pesticides under review, probably because historically the emphasis has been to demonstrate qualitatively the presence or absence of antithyroid effects with little or no attention devoted to developing dose–response information. It is anticipated that dose correlation data will be more readily available in studies submitted to the EPA in the future.

Mode of Action

Thyroid tumors in rodents seem to arise from different modes of action: mutagenic, antithyroid, or a combination of the two. It is as if mutagenic influences initiate the carcinogenic process, whereas the antithyroid influences mainly promote tumor formation by stimulating thyroid cell proliferation. Indirectly, antithyroid agents can also lead to an increase in mutations because there are more rounds of DNA synthesis, with each cell generation having some finite chance of mutation. *N*-Bis-(2-hydroxypropyl)nitrosamine is an example of a chemical that produces thyroid tumors exclusively through a mutagenic mode of action, as it is devoid of antithyroid activity (*4*). Trimethyl thiourea has antithyroid activity without mutagenic potential (*70,71*). The final common pathway for the various antithyroid modes of action is reduction in thyroid hormone levels and increase in the TSH level and its stimulation of the thyroid gland. Finally, a combination of mutagenic and various antithyroid means are also possible, as well as a mixture of different antithyroid modes. For instance, 4,4′-methylenedianiline and 3-methylcholanthrene are both mutagenic and antithyroid; the former is an inhibitor of thyroid peroxidase, while the latter induces liver microsomal enzymes and the metabolism and excretion of thyroid hormone (*1,62, 63,70,72*). Thiocyanate inhibits the active transport of iodide into the follicular cell as well, and it inhibits thyroid peroxidase; propylthiouracil is known to be antithyroid by two means, namely inhibition of both thyroid peroxidase and 5′-monodeiodinase (*44*). Diphenylthiohydantoin inhibits 5′-monodeiodinase and enhances hepatic thyroid hormone metabolism and excretion (*73*). Finally, polychlorinated biphenyls enhance the hepatic metabolism and excretion of thyroid hormones while also damaging follicular cells' ability to produce thyroid hormone (*45,63*). It is important to investigate how chemicals induce thyroid tumors in order to determine appropriate means to extrapolate from high to low dose and how to combine risks for chemicals with similar modes of action.

At least some thyroid tumor mode of action information is available for each of the 24 pesticides (Tables 4–7). All have some mutagenicity data, and 19 have information bearing on thyroid–pituitary disruption. Only 12 have sufficient information from which a possible thyroid carcinogenic mode of action can be inferred (Table 8). All 12 demonstrate an antithyroid action: 3 inhibit thyroid peroxidase, and 9 stimulate thyroid hormone metabolism and excretion.

Table 7. Reversibility of antithyroid effects upon cessation of pesticidal dosing

Pesticide	Treatment period (weeks)	Recovery period (weeks)	Thyroid growth[a]	Decreases in T_4 or T_3	Increases in TSH
Amitrole	4	4		Yes	
Ethiozin	13	4	Yes	No	
Ethylene thiourea	7	4	Yes	Yes	
Fenbuconazole	4	9	Yes	Yes	Yes
Fipronil	50	11		Yes	Yes
Pendimethalin	4	4	Yes	Yes	
Pentachloronitrobenzene	13	13	Yes	Yes	Yes
Pronamide	4	11	Yes	Yes	
Pyrimethanil	2	2	Yes	Yes	Yes
Thiazopyr	8	8–16	Yes[b]	Yes	Yes

Abbreviations: T_4, thyroxin; T_3, triiodothyronine; TSH, thyroid-stimulating hormone.

[a]Cellular hypertrophy, hyperplasia, and/or increased thyroid weight.
[b]Yes for histology and no for weight.

Table 8. Pesticidal mode of action of thyroid tumors

Antithyroid site of action	Pesticides	Comments
Mutagenic activity	Possibly acetochlor; less so for ethylene thiourea and etridiazole	
Inhibition of thyroid peroxidase activity	Amitrole, ethylene thiourea, mancozeb	
Inhibition of iodide pump	Amitrole, ethiozin, ethylene thiourea, pentachloronitrobenzene	Need data to differentiate from effect on thyroid peroxidase
Thyroid follicular cell injury		Not reported
Inhibition of thyroid hormone release		Not reported
Inhibition of 5′-monodeiodinase activity		Few studies
Enhancement of hepatic thyroid hormone metabolism and excretion	Acetochlor, clofentezine, fenbuconazole, fipronil, pendimethalin, pentachloronitrobenzene, prodiamine, pyrimethanil, thiazopyr	

In general, mutagenicity does not seem to be a major mode of action accounting for thyroid tumors. Evidence on acetochlor suggests that it may have two modes of action: it produces both gene mutations and structural chromosome aberrations in some but not all test systems, and it enhances thyroid hormone metabolism and excretion by the liver. None of the pesticides uniquely works through a mutagenic mode of action.

No pesticide has been investigated for all potential antithyroid sites of action. No information has been reported regarding some sites of action, namely, thyroid follicular cell injury and inhibition of thyroid hormone release; only a limited number of chemicals have been investigated for inhibition of 5'-monodeiodinase. Further experimental work is needed to help determine modes of action of pesticides. More than one mode of action may apply; methods are available to discern each of them. Future studies submitted to the EPA are expected to comply with EPA science policy (*12*).

Given the present assessment of pesticides as well as an evaluation of other chemicals, it is apparent that antithyroid activity is a common mode of thyroid carcinogenic action in rodents. Most of these chemicals appear to be inducers of hepatic microsomal enzymes. A smaller group includes thyroid peroxidase inhibitors, namely thionamides, some aromatic amines, and a few other agents, such as amitrole. It would seem that only a few chemicals might operate through other antithyroid modes of action: inhibition of iodine uptake, inhibition of thyroid hormone release from the thyroid gland, toxicity to the thyroid gland, and inhibition of 5'-monodeiodinase (*44,45,74*). When a new, unstudied chemical produces indication of thyroid hypertrophy or hyperplasia in repeat dosing studies, further work may be warranted in determining potential antithyroid activity and site of action before commencing more detailed investigations. Structural alerts and testing for mutagenicity can identify chemicals working by a DNA reactive mode of action.

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